Appl. No. 10/037,243, Filed January 4, 2002 Amendment Dated February 6, 2006 Reply to Office Action of August 9, 2005

<u>Claim Listing</u> This listing of claims will replace all prior versions and listings of claims in the application:

1 - 52: (cancelled)

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(currently amended) An expression vector, which vector 53. is optimized for use in prokaryotic cells, for enhancing the solubility and proper folding of an expressed protein. or polypeptide of interest said protein or polypeptide having an amino-terminus and a carboxyl-terminus, comprising a first nucleic acid sequence encoding a peptide extension, of 61 or fewer amino acid residues, the encoded peptide extension having a net negative charge ranging from 20 under physiological conditions which peptide extension comprises the carboxyl-terminal 57 amino acid residues of a T7 gene 10B protein; the expression vector further comprising a multiple cloning site for inserting, in-frame with said first nucleic acid sequence, a second nucleic acid sequence encoding the protein or polypeptide of interest, wherein expression of the nucleic acid sequences under physiological conditions yields a fusion protein consisting essentially of the encoded peptide

extension fused to the carboxyl-terminus of the protein or polypeptide of interest.

- 54. 62. (cancelled)
- 63. (currently amended) The expression vector of Claim 62
 53 wherein one or more of the amino acid residues of the
 encoded peptide extension are substituted, which
 substitutions result in the maintenance of a net negative
 charge between -2 and -20 for the encoded peptide
 extension.
- 64. (currently amended) The expression vector of Claim
 6253, wherein the encoded peptide extension is selected
 from the group consisting of: Peptide T7C (SEQ ID NO: 5),
 Peptide T7B (SEQ ID NO: 6), Peptide T7B1 (SEQ ID NO: 7),
 Peptide T7B2 (SEQ ID NO: 8), Peptide T7B3 (SEQ ID NO: 9),
 Peptide T7B5 (SEQ ID NO: 11), Peptide T7B6 (SEQ ID NO: 12),
 Peptide T7B7 (SEQ ID NO: 13), Peptide T7B8 (SEQ ID NO: 14),
 Peptide T7B9 (SEQ ID NO: 15), Peptide T7B10 (SEQ ID NO:
 16), Peptide T7B11 (SEQ ID NO: 17), Peptide T7B12 (SEQ ID
 NO: 18), Peptide T7B13 (SEQ ID NO: 19), Peptide T7A1 (SEQ
 ID NO: 21), Peptide T7A2 (SEQ ID NO: 22), Peptide T7A3 (SEQ
 ID NO: 23), Peptide T7A4 (SEQ ID NO: 24) and Peptide T7A5
 (SEQ ID NO: 25).

65 - 86 (cancelled)

- 87. (currently amended) The expression vector of Claim 62

 53 wherein the encoded peptide extension comprises the carboxyl-terminal 40 amino acid residues of the T7 gene 10B protein.
- 88. (currently amended) The expression vector of Claim 87 wherein one or more of the amino acid residues are substituted or deleted, which substitutions or deletions result in the maintenance of a net negative charge between -2 and -20 for the encoded peptide extension.
- 89. (currently amended) The expression vector orof Claim
 8788 wherein the encoded peptide extension comprises the
 carboxyl-terminal 18 amino acid residues of the T7 gene 10B
 protein and wherein one or more of the amino-acids are
 substituted, which said substitutions or deletions result
 in the maintenance of a net negative charge between -4 and
 -6 for the encoded peptide extension.

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- 90. (currently amended) An expression vector for enhancing the solubility and proper folding of an expressed protein or polypeptide of interest, which vector is optimized for use in bacterial cells, said protein or polypeptide having an amino-terminus and a carboxyl-terminus, comprising a first nucleic acid sequence encoding a 61 or fewer amino acid peptide extension comprising the carboxyl-terminal 57 amino acid residues of a bacteriophage T7 gene 10B protein, and further comprising a multiple cloning site for inserting, in-frame with said first nucleic acid sequence,
- a second nucleic acid sequence encoding the protein or polypeptide of interest, wherein expression of the nucleic acid sequences <u>under physiological conditions</u> yields a fusion protein consisting essentially of the encoded peptide extension fused to the carboxyl-terminus of the protein or polypeptide of interest.
- 91. (currently amended) The expression vector of Claim 90 wherein one or more of the amino acid residues are substituted or deleted, which substitutions or deletions result in a net negative charge between -2 and -20 under physiological conditions for the encoded peptide.

- 92. (previously presented) The expression vector of
 Claim 90 wherein the encoded peptide is selected from the
 group consisting of: Peptide T7C (SEQ ID NO: 5), Peptide
 T7B (SEQ ID NO: 6), Peptide T7B1 (SEQ ID NO: 7), Peptide
 T7B2 (SEQ ID NO: 8), Peptide T7B3 (SEQ ID NO: 9), Peptide
 T7B5 (SEQ ID NO: 11), Peptide T7B6 (SEQ ID NO: 12), Peptide
 T7B7 (SEQ ID NO: 13), Peptide T7B8 (SEQ ID NO: 14), Peptide
 T7B9 (SEQ ID NO: 15), Peptide T7B10 (SEQ ID NO: 16),
 Peptide T7B11 (SEQ ID NO: 17), Peptide T7B12 (SEQ ID NO:
 10 18), Peptide T7B13 (SEQ ID NO: 19), Peptide T7A1 (SEQ ID
 NO: 21), Peptide T7A2 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID
 NO: 23), Peptide T7A4 (SEQ ID NO: 24) and Peptide T7A5 (SEQ
 ID NO: 25).
 - 93. (currently amended) An expression vector, optimized for use in bacterial cells, for enhancing the solubility and proper folding of an expressed protein or polypeptide of interest, said protein or polypeptide having an aminoterminus and a carboxyl-terminus, comprising a first nucleic acid sequence encoding a peptide extension, which peptide extension is selected from the group consisting of: Peptide T7C (SEQ ID NO: 5), Peptide T7B (SEQ ID NO: 6),

- Peptide T7B3 (SEQ ID NO: 9), Peptide T7B5 (SEQ ID NO: 11), 10 Peptide T7B6 (SEQ ID NO: 12), Peptide T7B7 (SEQ ID NO: 13), Peptide T7B8 (SEQ ID NO: 14), Peptide T7B9 (SEQ ID NO: 15), Peptide T7B10 (SEQ ID NO: 16), Peptide T7B11 (SEQ ID NO: 17), Peptide T7B12 (SEQ ID NO: 18), Peptide T7B13 (SEQ ID NO: 19), Peptide T7A1 (SEQ ID NO: 21), Peptide T7A2 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 23), Peptide T7A4 (SEQ ID NO: 24) and Peptide T7A5 (SEQ ID NO: 25), and further comprising a multiple cloning site for inserting, in-frame with said first nucleic acid sequence, a second nucleic acid sequence encoding the protein or polypeptide of 20 interest, wherein expression of the nucleic acid sequences under physiological conditions yields a fusion protein consisting essentially of the encoded peptide extension fused to the carboxyl-terminus of the protein or polypeptide of interest.
 - 94. (new) The prokaryotic cell expression vector of claim 53 wherein the cell is E. coli.
 - 95. (new) The expression vector of Claim 90 wherein the bacterial cell is selected from the group consisting of E. coli, B. subtilis, and R. eutrophus.

- 96. (new) The expression vector of Claim 95 wherein the cell is E. coli.
- 97. (new) The expression vector of Claim 93 wherein the bacterial cell is selected from the group consisting of E. coli, B. subtilis, and R. eutrophus.
- 98. (new) The expression vector of Claim 97 wherein the cell in E. coli.